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### **An evaluation of a novel single tube method for extended genotyping of Human Papillomavirus**

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1   **Title: An evaluation of a novel single tube method for extended genotyping of Human**  
2   **Papillomavirus.**

3   **Running Title: Evaluation of novel single tube HPV genotyping assay**

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22 **Abstract:**

23 **Background:** The use of high-risk HPV testing for surveillance and clinical applications is  
24 increasing globally and it is important that tests are evaluated to ensure they are fit for  
25 purpose. In this study, the performance of a new HPV genotyping test -The  
26 Papilloplex® HR-HPV test- was compared to two well established genotyping tests.  
27 Preliminary clinical performance was also ascertained for the detection of CIN2+ in a  
28 disease-enriched retrospective cohort.

29 **Methods:** A panel of 500 cervical LBC samples with known clinical outcomes were tested  
30 by the Papilloplex® HR-HPV test. Analytical concordance was compared to two assays:  
31 Linear Array HPV Genotyping Test and Optiplex HPV Genotyping Test. Initial clinical  
32 performance for the detection for CIN2+ samples was performed and compared to that of two  
33 clinically validated HPV tests: the RealTime High Risk HPV test and Hybrid Capture 2 HPV  
34 Test.

35 **Results:** High agreement for HR-HPV was observed between the Papilloplex and LA and  
36 Optiplex HPV tests (97% and 95% respectively); with Kappa for HPV 16 and 18 being 0.90  
37 and 0.81 compared to the LA and 0.70 and 0.82 compared to Optiplex. The sensitivity,  
38 specificity, PPV and NPV of Papilloplex for detection of CIN2+ was 92%, 54%, 33% and  
39 96% respectively and was very similar to that observed with RealTime and HC2.

40 **Conclusion:** Papilloplex HR-HPV test shows similar analytical performance to two HPV  
41 genotyping tests at the level of HR-HPV and type specific level. Preliminary data on clinical  
42 performance look encouraging although further longitudinal studies within screening  
43 populations are required to confirm this.

## 44 **Introduction**

45 The use of high-risk HPV (HR-HPV) testing for the identification of women at risk of  
46 developing cervical cancer and for the management of women who have received treatment  
47 is increasing globally (1). Additionally, type specific HPV detection methods are valuable  
48 both for epidemiological studies and as a triage for primary HR-HPV infection (2). There are  
49 now a wide variety of commercially available HPV tests (3) which vary in terms of detection  
50 chemistry, complexity, type range, throughput and required equipment. While a component  
51 have been clinically validated for use in primary HPV screening through assessment  
52 according to internationally accepted criteria, or used extensively in longitudinal research and  
53 surveillance endeavours; peer reviewed evidence on the analytical and/or clinical  
54 performance of several tests is lacking.

55 The Papilloplex<sup>®</sup> HR-HPV test (Genefirst, UK) is a commercially available HPV genotyping  
56 test that performs quantitative multiplex detection of 14 HR-HPV types, together with an  
57 endogenous human control target, in a single tube (4). Based on Multiplex Probe  
58 Amplification (MPA) technology, the assay utilises differing melting curve profiles to allow  
59 the differentiation of up to six targets per fluorescence channel within a real-time assay (4).  
60 The test is compatible with real-time polymerase chain reaction (PCR) equipment commonly  
61 used in clinical and research laboratories and so does not require a specific locked-down  
62 platforms.

63 Here we present results from an evaluation of the Papilloplex HR-HPV assay where its  
64 performance is compared to two qualitative, broad spectrum, extended genotyping assays –  
65 the Linear Array (LA) HPV Genotyping Test (Roche Molecular Systems Inc., Alameda, CA,  
66 USA) and the Optiplex HPV Genotyping Kit (formerly Multiplex HPV Genotyping Kit,  
67 DiaMex, Heidelberg, Germany). Preliminary insight into clinical performance of the assay is  
68 also presented through its ability to detect CIN2+ in a disease-enriched sample compared to

69 two well established clinically validated HPV assays – Hybrid Capture 2 (HC2) HPV DNA  
70 Test (Qiagen Gaithersburg, Inc., MD, US) and the RealTime High Risk HPV test (Abbott  
71 Molecular, Des Plaines, IL, USA).

72

## 73 **Results**

### 74 **Overall HR-HPV positivity in the cohort**

75 The study cohort consisted of 500 Thinprep<sup>®</sup> liquid based cytology (LBC) samples with  
76 known cytology and histology results (Table 1). The sample cohort of 500 was split into two  
77 extraction methods (250 extracted using manual QiaAmp DNA mini kit and 250 using  
78 automated Nuclisens EasyMag system). The concordance of Papilloplex at overall HR-HPV  
79 level and type specific level with LA and Optiplex showed no significant differences based  
80 on extraction chemistry (data not shown). The whole study cohort was therefore used for  
81 further analysis. Overall HR-HPV positivity for the genotyping tests and the clinically  
82 validated tests was similar: 58.4% for Papilloplex, 57.2% for LA, 56.4% for Optiplex, 56.2%  
83 for RealTime and 58.6% for HC2 (Table 2).

### 84 **Agreement between assays**

85 Agreement of overall HR-HPV positivity between Papilloplex and the two extended  
86 genotyping tests is shown in Table 3. High proportional agreement of 97% (95% CI- 95-98)  
87 was observed between Papilloplex and LA. Similarly, high proportional agreement of 95%  
88 (95% CI- 92-97) was observed between Papilloplex and Optiplex.

89 Type specific concordance(s) between the Papilloplex and the two genotyping assays for HR-  
90 HPV types 16,18,31,33,35,39,45,51,52,56,58,59,66 and 68 are shown in Table 4. Two by two  
91 tables for each type detected by Papilloplex (vs comparator test) are also presented in  
92 Supplementary Data (Table S1). When comparing the Papilloplex to the Optiplex test there

93 was at least “substantial” agreement (defined according to a kappa of 0.61 to 0.80) for all  
94 types except HPV 68 (0.548). The equivalent comparison of Papilloplex to LA showed at  
95 least substantial agreement (defined according to a kappa of 0.61 to 0.80) for all types except  
96 HPV 68 (0.573) and HPV 59 which at a Kappa of 0.614 was at the lower end of substantial  
97 agreement. Papilloplex detected fewer samples as positive for HPV 16 (N=98) compared to  
98 both LA (N=108) and Optiplex (N=146). Similarly for HPV 59, Papilloplex detected fewer  
99 samples as positive (N=20) compared to LA (N= 73) and Optiplex (N=28) which is reflected  
100 in the aforementioned Kappa value. Conversely, Papilloplex detected a higher number of  
101 HPV 31 (N=64) infections compared to LA (N=54) and Optiplex (N=40), and a higher  
102 number of HPV 33 (N=44) infections vs Optiplex (N=36). Papilloplex also detected a higher  
103 number of HPV 56 (N=32) infections compared to LA (N=22) but this was lower than those  
104 detected by Optiplex (N=43) (Table S1).

#### 105 **Clinical performance for detection of Cervical intraepithelial neoplasia 2 or worse** 106 **(CIN2+)**

107 Of the 500 samples in the panel 87 were associated with CIN2+. Sensitivity, specificity,  
108 positive predictive value (PPV) and negative predictive value (NPV) of the Papilloplex test  
109 for the detection of CIN2+ is summarised in Table 5, with values of 92%, 54%, 33% and  
110 96% respectively. These values were similar to the clinical performance of the HC2 and  
111 RealTime assays.

#### 112 **Discussion**

113 Papilloplex HR- HPV test is a single tube test for the quantitative multiplex detection of 14  
114 HR-HPV types, together with an endogenous human control target. This study provides the  
115 first analytical assessment of the Papilloplex test compared to two commercially available  
116 HPV tests that offer extended genotyping capability: LA and Optiplex. Further, to gain

117 insight into the potential clinical performance of the assay a preliminary evaluation was  
118 undertaken to determine its ability to detect CIN2+ in a disease enriched population.

119 Papilloplex showed high concordance to Optiplex and LA at the level of overall HR-HPV  
120 positivity with a proportional agreement of 95-97% and kappa of 0.90- 0.93. Type specific  
121 proportional agreement for all 14 HR-HPV types covered by Papilloplex was generally high  
122 although there were some type specific differences. Papilloplex showed moderate  
123 concordance to LA and Optiplex for HPV 16 and 59, detecting less infections and clearly,  
124 HPV 16 is an important type for both epidemiological and clinical applications. On the other  
125 hand, Papilloplex detected more HPV 31 infections compared to both comparator genotyping  
126 tests. Type specific differences between genotyping tests have been reported previously (5)  
127 and such differences are perhaps inevitable given the range of chemistries available.

128 Nevertheless, these data reinforce the notion that for longitudinal surveillance exercises (in  
129 which monitoring prevalence and trends of HPV types is important), consistent use of the  
130 same test is important to avoid real changes being confounded by test chemistry.

131 Furthermore, it is notable that the clinical performance of the Papilloplex assay was similar to  
132 that of two well established clinically validated tests indicating that type-specific differences  
133 (including for HPV 16) may not have significant implications for the detection of disease.

134 This said, we accept that the clinical evaluation performed in this study was preliminary and  
135 that the sample used was enriched in nature and did not represent women from a cross section  
136 of the screening population. Consequently, the clinical performance observed in this study,  
137 will not be representative of performance in a screening population. Nevertheless,  
138 determining initial sensitivity (the key measure of performance for screening applications) of  
139 a novel HPV test for CIN2+ using a sample with high disease-prevalence has precedent (6, 7)  
140 and arguably showing performance relative to that of an assay in which clinical efficacy has  
141 been demonstrated also has value, even at an early stage. Furthermore, future clinical

142 validation of the test which builds on the present work but involves a longitudinal screening  
143 population and assessment according to internationally recognised validation criteria is  
144 planned (8, 9).

145 The variety of HPV tests available with their different scope and capabilities provides users  
146 with options to choose the most appropriate test for a particular context and population.  
147 Papilloplex HPV is a single-tube assay that identifies 14 HR-HPV types. The ability to  
148 perform individual genotyping within a single closed-tube format reduces time and risk of  
149 contamination associated with more “open” genotyping systems. The assay is amenable to  
150 several DNA extraction chemistries, requires a low amount of input DNA and can be  
151 performed with existing real-time 96 well PCR platforms that are available in routine  
152 research and clinical laboratories. In terms of analytical performances we have shown that  
153 this assay compares favourably to existing more established extended genotyping assays.  
154 While initial data on clinical performance is encouraging, further longitudinal assessments  
155 will determine its potential use for screening and disease management.

156

## 157 **Material and methods**

### 158 **Samples and approvals**

159 A total of 500 liquid based cytology samples (LBCs) were obtained from the Scottish HPV  
160 Archive ([www.shine.mvm.ed.ac.uk/archive](http://www.shine.mvm.ed.ac.uk/archive)) which is a biobank designed to support HPV  
161 Research. The East of Scotland Research Ethics Service has given generic approval to the  
162 Scottish HPV Archive as a Research Tissue Bank (REC Ref 11/AL/0174) for HPV related  
163 research on archived samples. The Scottish HPV Archive is also registered with National  
164 Research Scotland (NRS) Lothian Bioresource. Samples were made available for the present



165 project through application to the archive steering committee (HPV Archive Application Ref  
166 0016).

167 The samples used for the study included 473 samples collected from women attending their  
168 first routine smear at the age of 20 in Scotland, supplemented by 27 samples from women  
169 attending colposcopy clinics due to abnormal cytology (in order to enrich for CIN2+).  
170 Routine cytology classification was as per British Society for Clinical Cytology criteria (10).  
171 Cytology results were classed as negative (for any abnormality), low grade (borderline  
172 squamous changes, koilocytosis, and low grade dyskaryosis) and high grade (which includes  
173 moderate and severe dyskaryosis). Subsequent cytology and histology results were obtained  
174 through data linkage via Information Services Division, Scotland and samples were classified  
175 as 2x cytology negative (with 2 subsequent negative cytology results at least 1 year apart),  
176  $\leq$ CIN1 or CIN2+ (Table 1). Samples had originally been collected between 2010 and 2012  
177 and stored in the archive at -80°C.

#### 178 **HPV DNA testing**

179 Samples were retrieved and aliquots prepared for HPV testing with Papilloplex HR-HPV test,  
180 HC2, Optiplex HPV genotyping test, LA and RealTime HR-HPV test. Papilloplex test was  
181 performed in Genefirst laboratories (Oxford, UK). All other tests were performed at the  
182 Scottish HPV Reference Laboratory and HPV Research Group (Edinburgh). All tests were  
183 performed according to manufacturer's instructions although a brief description of assay  
184 characteristics is provided in Table 2 and a detailed description of the Papilloplex HR-HPV  
185 test is provided in the next section. The Optiplex genotyping test has been used for  
186 longitudinal immunisation surveillance in Scotland (11–13) and has been adjudicated as  
187 proficient for detection of HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68  
188 according to the last three consecutive WHO laboratory network (WHO LabNet) HPV DNA

189 proficiency schemes (when testing was performed in Edinburgh). LA is also associated with  
190 good performance on WHO LabNet proficiency panels as outlined in Eklund et al (2014)  
191 where it was the most frequently applied assay to the scheme (7).

#### 192 **Papilloplex HPV test**

193 The Papilloplex HR-HPV test was performed on DNA extracted using two different methods.  
194 Half the samples were extracted using QiaAmp DNA mini kit (Qiagen, Germany) and half  
195 using automated Nuclisens EasyMag system (BioMérieux, France). The method of extraction  
196 was randomly allocated to samples.

197 A total of 2µl of DNA was added to the PCR amplification reaction mix (18µl) containing  
198 buffer (dNTPs and Mg<sup>2+</sup>), master mix (Taq polymerase, UNG enzyme and dUTP) and  
199 working mix (primers and probes) to obtain a final volume of 20µl per PCR reaction. The  
200 PCR was performed on ABI 7500 Fast Real-Time PCR Systems (Applied Biosystems,  
201 Warrington, UK). The thermal profile was set to: Amplification stage 1 (50°C for 2 min,  
202 followed by 95°C for 3 min), amplification stage 2 (9 cycles of 95°C for 6 sec, followed by  
203 66°C for 45 sec), and amplification stage 3 (42 cycles of 95°C for 3 sec, followed by 60°C  
204 for 33 sec, and 63°C for 15 sec). Fluorescence measurements in the ROX, FAM, HEX (JOE),  
205 and CY5 channels were recorded during step 2 of amplification stage 3 (60°C for 33 sec). A  
206 pre-set dissociation stage (stage 4) was included following the final PCR cycle of  
207 amplification (stage 3). The post-amplification melting profile protocol comprised of 95°C  
208 for 15 sec, 25°C for 1 min, 75°C for 15 sec, and 60°C for 15 sec. The fluorescence emission  
209 data was continually collected during the temperature increase. The negative derivative of the  
210 emission reading, with respect to temperature, was plotted against the temperature to form  
211 melting curves (per fluorescent channel) generated during the dissociation stage of the  
212 reaction (from 25°C to 75°C).

213 For consistency between experiments, the following threshold values for Ct determination  
214 were set (ROX: 100,000; FAM: 100,000; HEX: 25,000 and CY5: 50,000). For each sample,  
215 the internal control (CY5 detection channel) and all fourteen HR-HPV types, corresponding  
216 to the ROX (HR-HPV types: 33, 35, 45, 51, 56, and 66), FAM (HR-HPV types: 16, 18, 31,  
217 52, and 59) and HEX channel (HR-HPV types: 39, 58, 68) were simultaneously evaluated.  
218 Samples were considered positive for HR-HPV DNA types if a Ct value was < 38 for cellular  
219 DNA and < 36 in any of the ROX, FAM and HEX fluorescent channels. A sample was  
220 considered invalid if the Ct value of cellular DNA was >38. The change in the characteristic  
221 melting profile(s) in the sample was compared to the negative control reference melting  
222 profile to identify the genotypes present. Samples were tested in batches of 96 samples  
223 (including controls) per reaction.

## 224 **Analysis**

### 225 **HR-HPV concordance of the Papilloplex with comparator tests**

226 Type specific positivity for each HR-HPV type included in Papilloplex was compared to the  
227 Optiplex and LA. Concordance, proportional agreement with accompanying 95% confidence  
228 intervals (CI) have been presented along with kappa statistics and McNemar's test. The  
229 Papilloplex was also compared to the above tests at the level of HR-HPV positivity (for the  
230 types covered by Papilloplex only).

231 **Assessment of preliminary clinical performance**

232 Clinical performance of the Papilloplex test was measured as sensitivity, specificity, positive  
233 predictive value and negative predictive value for the detection of cervical CIN2+ with 95%  
234 CI's around the percentages. The clinical performance of the HC2 and RealTime HPV test  
235 was also performed and presented alongside the Papilloplex results. Disease cases were  
236 defined as CIN2+ (n=87), whereas no disease was defined as histologically confirmed CIN1  
237 or less or a sample being associated with two consecutive negative cytology results at least 1  
238 year apart (n=349). Pathology data was incomplete to allow this categorisation for 64/500  
239 samples so clinical performance assessment was performed on 436 samples.

240

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252 **References**

- 253 1. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. 2011.  
254 Human Papillomavirus Testing in the Prevention of Cervical Cancer. *JNCI J Natl*  
255 *Cancer Inst* 103:368–383.
- 256 2. Schiffman M, Boyle S, Raine-Bennett T, Katki HA, Gage JC, Wentzensen N, Kornegay  
257 JR, Apple R, Aldrich C, Erlich HA, Tam T, Befano B, Burk RD, Castle PE. 2015. The  
258 role of human papillomavirus (HPV) genotyping in cervical cancer screening: A large-  
259 scale evaluation of the cobas HPV test. *Cancer Epidemiol Biomark Prev Publ Am Assoc*  
260 *Cancer Res Cosponsored Am Soc Prev Oncol* 24:1304–1310.
- 261 3. Poljak M, Kocjan BJ, Oštrbenk A, Seme K. 2016. Commercially available molecular  
262 tests for human papillomaviruses (HPV): 2015 update. *J Clin Virol* 76:S3–S13.
- 263 4. Fu G, Miles A, Alphey L. 2012. Multiplex Detection and SNP Genotyping in a Single  
264 Fluorescence Channel. *PLOS ONE* 7:e30340.
- 265 5. Rebolj M, Preisler S, Ejegod DM, Rygaard C, Lynge E, Bonde J. 2014. Disagreement  
266 between Human Papillomavirus Assays: An Unexpected Challenge for the Choice of an  
267 Assay in Primary Cervical Screening. *PLoS ONE* 9.
- 268 6. Venturoli S, Leo E, Nocera M, Barbieri D, Cricca M, Costa S, Santini D, Zerbini M.  
269 2012. Comparison of Abbott RealTime High Risk HPV and Hybrid Capture 2 for the  
270 detection of high-risk HPV DNA in a referral population setting. *J Clin Virol Off Publ*  
271 *Pan Am Soc Clin Virol* 53:121–124.
- 272 7. Eklund C, Forslund O, Wallin K-L, Dillner J. 2014. Global improvement in genotyping  
273 of human papillomavirus DNA: the 2011 HPV LabNet International Proficiency Study.  
274 *J Clin Microbiol* 52:449–459.

- 275 8. Meijer CJLM, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, Arbyn M,  
276 Bosch FX, Cuzick J, Dillner J, Heideman DAM, Snijders PJF. 2009. Guidelines for  
277 human papillomavirus DNA test requirements for primary cervical cancer screening in  
278 women 30 years and older. *Int J Cancer J Int Cancer* 124:516–520.
- 279 9. Arbyn M, Depuydt C, Benoy I, Bogers J, Cuschieri K, Schmitt M, Pawlita M, Geraets  
280 D, Heard I, Gheit T, Tommasino M, Poljak M, Bonde J, Quint W. 2016. VALGENT: A  
281 protocol for clinical validation of human papillomavirus assays. *J Clin Virol Off Publ*  
282 *Pan Am Soc Clin Virol* 76 Suppl 1:S14-21.
- 283 10. Achievable standards, Benchmarks for reporting, and Criteria for evaluating cervical  
284 cytopathology. <http://www.cancerscreening.nhs.uk/cervical/publications/nhscsp01.html>.
- 285 11. Kavanagh K, Pollock KG, Cuschieri K, Palmer T, Cameron RL, Watt C, Bhatia R,  
286 Moore C, Cubie H, Cruickshank M, Robertson C. 2017. Changes in the prevalence of  
287 human papillomavirus following a national bivalent human papillomavirus vaccination  
288 programme in Scotland: a 7-year cross-sectional study. *Lancet Infect Dis* Sep 28. pii:  
289 S1473-3099(17)30468-1.
- 290 12. Kavanagh K, Pollock KGJ, Potts A, Love J, Cuschieri K, Cubie H, Robertson C,  
291 Donaghy M. 2014. Introduction and sustained high coverage of the HPV bivalent  
292 vaccine leads to a reduction in prevalence of HPV 16/18 and closely related HPV types.  
293 *Br J Cancer* 110:2804–2811.
- 294 13. Kavanagh K, Sinka K, Cuschieri K, Love J, Potts A, Pollock KG, Cubie H, Donaghy M,  
295 Robertson C. 2013. Estimation of HPV prevalence in young women in Scotland;  
296 monitoring of future vaccine impact. *BMC Infect Dis* 13:519.
- 297

298 **Results Tables**

<b>Underlying Cytology</b>	<b>N (%)</b>
Negative	266 (53.2)
Low grade dyskaryosis	156 (31.2)
High grade dyskaryosis	66 (13.2)
Unknown	12 (2.4%)
Total	500
<b>Underlying Histology</b>	
No histology performed (2 x Negative cytology)	263 (52.6)
≤CIN1	86 (17.2)
CIN2+	87 (17.4)
Histology information incomplete	64 (12.8)

299 **Table 1: Cervical pathology associated with study population. Note that clinical**  
300 **performance assessment was performed on 436 samples. Samples with incomplete**  
301 **histology was not included in this analysis.**

Test	Detection technology	High-risk types identified by the test	Low-risk types identified by the test	High-risk positive (N, %)	High-risk + Low-risk positive (N)
Papilloplex HR-HPV test	Real-time PCR with individual genotyping	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68		292 (58.4%)	
RealTime HR-HPV test	Real-time PCR with partial genotyping	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68		281 (56.2%)	
Hybrid Capture 2 (HC2)	Target amplification followed by Sandwich capture assay	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 [not 66]		293 (58.6%)	
Linear Array HPV Genotyping test	Target amplification followed by hybridisation	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108	286 (57.2%)	340 (68.0%)
Optiplex HPV genotyping test	Target amplification followed by luminex detection	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	6, 11, 26, 42, 43, 44, 53, 70, 73, 82	282 (56.4%)	321 (64.2%)

**Table 2: Description of assays used in the study with the detection technology, types**

**covered and prevalence of HPV in the study population.**



Linear Array (LA) HPV Genotyping test						
		NEG	POS	Proportional agreement	Kappa	McNemars test: p-value
Papilloplex HR-HPV test	NEG	203	5	97%	0.934	0.210
	POS	11	281	(95, 98)		
Optiplex HPV genotyping test						
		NEG	POS	Proportional agreement	Kappa	McNemars test: p-value
Papilloplex HR-HPV test	NEG	200	8	95%	0.894	0.076
	POS	18	274	(92, 97)		

311 **Table 3: Overall agreement between Papilloplex HR-HPV test and comparator tests.**

312 **Concordance between the samples are indicated and proportional agreement with 95%**

313 **CI (in brackets), kappa and McNemar's test p-value are listed.**

314

	HPV type	Optiplex HPV test	Linear array HPV test	HPV type	Optiplex HPV test	Linear array HPV test
Proportional agreement	16	89% (86, 91)	97% (95, 98)	51	98% (96, 99)	98% (97,99)
Kappa		0.7	0.902		0.879	0.914
McNemars test: p-value		<0.001	0.021		1	0.727
Proportional agreement	18	97% (95, 98)	97% (95, 98)	52	96% (94, 97)	*
Kappa		0.822	0.809		0.811	*
McNemars test: p-value		0.286	0.077		0.664	*
Proportional agreement	31	95% (93, 97)	97% (95, 98)	56	97% (95, 98)	98% (96, 99)
Kappa		0.744	0.846		0.784	0.805
McNemars test: p-value		<0.001	0.021		0.007	0.002
Proportional agreement	33	98% (97, 99)	99% (97, 99)	58	98% (96, 99)	98% (97, 99)
Kappa		0.966	0.91		0.811	0.886
McNemars test: p-value		0.008	0.453		0.146	0.727
Proportional agreement	35	99% (98, 100)	100% (99, 100)	59	98% (96, 99)	95% (93, 97)
Kappa		0.774	0.907		0.738	0.614
McNemars test: p-value		0.125	1		0.039	<0.001
Proportional agreement	39	97% (96, 99)	98% (96, 99)	66	99% (97, 100)	99% (97, 99)
Kappa		0.851	0.937		0.915	0.908
McNemars test: p-value		0.774	0.388		1	0.016
Proportional agreement	45	99% (96, 99)	99% (98, 100)	68	98% (97, 99)	98% (96, 99)
Kappa		0.867	0.924		0.548	0.573
McNemars test: p-value		1	1		0.07	1

315 **Table 4: Type specific agreement of Papilloplex with Optiplex and Linear array (LA)**  
316 **HPV tests. Proportional agreement with 95% CI (in brackets), kappa and McNemar's**  
317 **test p-value are indicated. \*- Linear Array (LA) is unable to identify HPV-52 status in**  
318 **samples also positive for HPV33, HPV35, and/or HPV58. Results for HPV-52 is therefore**  
319 **not presented.**

	<b>Papilloplex HR- HPV test</b>	<b>Hybrid Capture 2 (HC2)</b>	<b>RealTime HR- HPV test</b>
Sensitivity	92% (84, 97)	91% (83, 96)	91% (83, 96)
Specificity	54% (48, 59)	54% (48, 59)	56% (50, 61)
PPV	33% (27, 39)	33% (27, 39)	34% (28, 40)
NPV	96% (93, 99)	96% (92, 98)	96% (92, 98)

320 **Table 5: Clinical performance of HPV tests for detection of CIN2+. Sensitivity,**  
321 **Specificity, Positive predictive value (PPV) and Negative Predictive value (NPV) along**  
322 **with 95% CI (in brackets) are indicated.**